***Temporal and spatial variations in the abundance and population structure of the spined loach (*Cobitis taenia*), a scarce fish species: implications for condition assessment and conservation***

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**ABSTRACT**

1. Effective conservation of protected species requires accurate estimates of the status of their populations. In the UK, this led to the production of a series of sampling protocols to establish the status of designated species against predetermined conservation objectives: a process known as ‘condition assessment’. Condition assessments involve comparisons of various parameters, invariably including abundance and/or population structure, of the target species against criteria that are judged to be indicative of viable populations.
2. This study investigated temporal and spatial variations in the abundance and population structure of spined loach (*Cobitis taenia*), a scarce species indigenous to Europe and central Asia. Specifically, the study compared the density, number of age classes and percentage contribution of the 0+ year age class of spined loach between day and night, months, years and locations.
3. There were marked diel, seasonal, annual and spatial variations in the density, number of age classes and percentage contribution of 0+ year spined loach. Such phenomena are important because monitoring programmes conducted at inappropriate times of day or year, or with insufficient frequency or geographical coverage, could lead to inaccurate assessments of the condition of protected populations and, consequently, to inadequate conservation measures. Notwithstanding, there were few impacts on the condition assessments of the spined loach populations because at least one of the parameters invariably failed to satisfy the population condition assessment criteria.
4. A prerequisite for successful conservation is an effective monitoring programme. It is therefore essential that surveys to assess the condition of populations of protected

species are designed with due consideration of their diel behaviour, breeding season, life span and habitat use. It is recommended that the monitoring protocol and condition assessment criteria for spined loach are amended, and that surveys are conducted: (1) by trawling; (2) in late summer; and (3) at least every 3-4 years.

KEY WORDS: conservation evaluation, ecological status, fish, floodplain, monitoring, river, wetland

## INTRODUCTION

Effective conservation of protected species requires accurate estimates of the status of their populations. In the UK, this led to the production of a series of sampling protocols (see Life in UK Rivers, 2003; Hurford *et al*., 2010) to establish the status of designated species against predetermined conservation objectives: a process known as 'condition assessment'. Condition assessments involve comparisons of various parameters, invariably including abundance and/or population structure, of the target species against criteria that are judged to be indicative of viable populations (Joint Nature Conservation Committee, 2005; Nunn *et al*., 2008; Cowx *et al*., 2009, 2010; Harvey *et al*., 2010). Estimates of the abundance and population structure of some species can vary on a temporal or spatial basis (Copp, 2008), however, which could have implications for the condition assessment and conservation of their populations. Sampling strategies must therefore be able to detect changes in both temporal and spatial structure relating to species distributions and abundances if conservation and management is to be effective (Cowx *et al*., 2009, 2010; Reynolds *et al*., 2011; Rolls *et al*., 2013).

The spined loach (*Cobitis taenia* L.) occurs across almost the whole of Europe and central Asia (Bohlen and Ráb, 2001; Janko *et al*., 2007), but is endangered in many European countries (Kotusz, 1996) and regarded as threatened in the UK (Maitland and Lyle, 1991; Joint Nature Conservation Committee, 2010). In mainland Europe, the situation is complicated by a propensity of the species to develop mixed diploid-polyploid populations, whereas it is believed that only pure diploid populations occur in the UK (Bohlen and Ráb, 2001; Boroń *et al*., 2003; Culling *et al*., 2006; Janko *et al*., 2007). The species is listed in Appendix III of the Bern Convention and Annex II of the

EC Habitats Directive (92/43/EEC) on the Conservation of Natural Habitats and of Wild Fauna and Flora, the latter of which requires European Member States to ensure its favourable conservation status through the protection of viable populations in designated Special Areas of Conservation (SACs) and throughout its range. The aim of this study was to investigate temporal and spatial variations in the abundance and population structure of spined loach. Specifically, the objectives were to compare the density, number of age classes and percentage contribution of the 0+ year age class of spined loach between day and night, months, years and locations. The rationale was that temporal and spatial variations in the abundance and population structure of spined loach could lead to inaccurate assessments of the condition of their populations and, consequently, to inadequate conservation measures. The implications of temporal and spatial variations in abundance and population structure for the conservation of spined loach, an endangered or threatened species across much of its range, are discussed, and improvements to the protocol used for condition assessment in the UK are suggested.

## METHODS

### Study area

The study was carried out at 21 sites on the River Trent, eight on the River Ancholme and 150 on the River Glen Counter and Gravel Drains, England (Figure 1). The Trent has a catchment area of 10 500 km$^2$ and is one of only two major rivers in the UK that support populations of spined loach, the other being the Great Ouse (Wheeler, 1977; Robotham, 1978; Nunn *et al*., 2003). The species is also native to a number of smaller rivers in eastern England, namely the Welland, Nene and Witham, but is believed to have been accidentally introduced to the River Ancholme and Suffolk Stour via water-transfer schemes (Davies *et al*., 2004; Copp and Wade, 2006). The spined loach is the primary reason that the River Glen Counter Drain, in the Welland catchment, was notified as a SAC.

### Sampling strategy and data collection

The Trent (1999-2012) and Ancholme (2008-2011) were surveyed monthly during daylight using a micromesh seine net (25-m long, 3-m deep, 3-mm hexagonal mesh), which was set parallel to the bank by wading. This net captures fishes as short as 5 mm, and is often a very effective method of catching large numbers of small-bodied individuals (Cowx *et al*., 2001). In addition, a boating marina connected to the lower River Trent was surveyed every 3 h during eight 24-h periods (June-July 2009, May-July 2010). These surveys were conducted to investigate temporal (diel, seasonal and annual) variations in the abundance and population structure of spined loach. Sampling areas (range 40-108 m$^2$) were calculated as a product of the length and width of the water column enclosed by the net.

The Counter and Gravel Drains were surveyed during daylight in October 2012 using an epibenthic trawl (1-m wide, 0.5-mm-meshed cod-end), which was pulled by hand at a constant speed (~0.25 m $s^{-1}$) using a 6-m rope (6-m transects). The trawl was used to collect numerous small samples, to investigate spatial variations in the abundance and population structure of spined loach. The sampling area (6 $m^2$) was calculated as a product of the trawl width and transect length. In addition, ten nocturnal samples, and three diurnal and three nocturnal seine samples, were collected to allow a comparison of gears between day and night. All spined loach were measured (total length, $L_T$, nearest mm) and immediately returned to the water.

**Data analysis**

According to the condition assessment protocol that is currently used in the UK, spined loach populations must meet three criteria to achieve 'favourable condition': (1) a density of at least 0.1 $m^{-2}$; (2) at least three age classes; and (3) a high percentage, preferably at least 50%, of 0+ year individuals (Joint Nature Conservation Committee, 2005). A failure to satisfy any of the criteria results in an 'unfavourable condition' status (Joint Nature Conservation Committee, 2005). For each sample, the abundance of spined loach was therefore converted to density (no. $m^{-2}$) by dividing the numbers captured by the area surveyed. Diel variations in abundance were investigated by plotting density over time for each 24-h survey; this is relevant because the spined loach is primarily a nocturnal species. Maximum and mean densities were calculated for diurnal (08:00, 11:00, 14:00, 17:00, 20:00) and nocturnal (23:00, 02:00, 05:00) samples (all surveys combined, including zero catches; $n$ = 64), and then compared using a

Mann-Whitney *U*-test. In addition, the relative frequency of occurrence (%*O*) and relative abundance (%*A*) of spined loach was calculated: $\%O = (O_d\ O_n^{-1}) \times 100$ and $\%A = (A_d\ A_n^{-1}) \times 100$, where $O_d$ was the number of diurnal samples (all surveys combined) that contained spined loach, $O_n$ was the number of nocturnal samples (all surveys combined) that contained spined loach, $A_d$ was the mean density of spined loach in diurnal samples (all surveys combined), and $A_n$ was the mean density of spined loach in nocturnal samples (all surveys combined). Mean densities of spined loach in diurnal and nocturnal trawl catches were compared using an independent samples *t*-test.

Mean densities of spined loach in the River Trent were calculated for each month (all surveys combined, including zero catches; $n$ = 172) from January-November 2006 (restricted to the Trent in 2006 for brevity) and compared using a Kruskal-Wallis test, to investigate seasonal variations in abundance; this is relevant because the current monitoring protocol states that surveys should be conducted in the autumn/winter, after the spawning period (Joint Nature Conservation Committee, 2005). In addition, mean autumn/winter (September-February) densities of spined loach in the Rivers Trent and Ancholme were calculated for each year (all surveys combined, including zero catches; $n$ = 341) and compared using a Kruskal-Wallis test, to investigate annual variations in abundance; this is relevant because the reporting frequency for SAC species is 6 years (Joint Nature Conservation Committee, 2005). GIS software was then used to map spatial variations in spined loach abundance, and mean densities were compared between the Counter and Gravel Drains (all samples combined, including zero catches; $n$ = 150) using a Mann-Whitney *U*-test, and between sections of the Counter Drain (sites 1-10, 11-20, 21-30, 31-40 and 41-50; $n$ = 50) using a Kruskal-Wallis test. Finally, mean densities of spined loach in trawl and seine catches were compared using a Mann-Whitney *U*-test.

Length distributions (2-mm $L_T$ classes) were derived to facilitate interpretation of the age structure of the spined loach populations (i.e. to determine the number of age classes present and the percentage contribution of 0+ year individuals). When catches were sufficient, modal groups (≈ age classes) were identified using modal progression analysis (Bhattacharya, 1967; Gayanilo *et al*., 1997) in FiSAT (FAO/ICLARM Stock Assessment Tools), otherwise the *minimum* number of age classes present was estimated by eye (see Nunn *et al*., 2008). The length distributions were used to examine diel, seasonal, annual and spatial variations in the structure of the spined loach populations, and were compared between the Counter and Gravel Drains using a two-sample Kolmogorov-Smirnov test (Dytham, 2003). The results were interpreted with reference to the criteria, described earlier, that are judged to be indicative of viable populations and that are used for condition assessment by the conservation bodies in the UK (Joint Nature Conservation Committee, 2005).

## RESULTS

### Diel variations

A total of 3573 spined loach, ranging from 13 to 97 mm $L_T$, was captured during the study. There were marked diel variations in the abundance of spined loach, with densities in seine catches generally being low during the day and peaking at night (Figure 2). Indeed, densities were often zero during the day (58% of diurnal samples; max. = 0.73 $m^{-2}$) but increased at night (max. = 1.55 $m^{-2}$), when densities were up to an order-of-magnitude higher (Mann-Whitney *U*-test, $U = 219.500$, $n = 64$, $P < 0.001$). The relative frequency of occurrence and relative abundance of spined loach in diurnal samples was 89% and 15% of nocturnal samples, respectively. The density of spined loach satisfied the criterion for 'favourable condition' (>0.10 $m^{-2}$) at night (mean ± S.D. from all surveys combined = 0.30 ± 0.47 $m^{-2}$) but not during the day (mean ± S.D. from all surveys combined = 0.05 ± 0.13 $m^{-2}$). The poor diurnal sampling efficiency meant that there were also apparent diel differences in spined loach 'population structure' (i.e. fewer age classes were captured during the day than at night). Notwithstanding, despite the diel variations in the density and age structure of spined loach catches, there were no differences in the condition of the population based on diurnal and nocturnal surveys because at least one of the parameters always failed to satisfy the assessment criteria (Table 1). In contrast to the seine catches, there was no significant difference in the abundance of spined loach in diurnal (mean ± S.D. = 0.06 ± 0.16 $m^{-2}$) and nocturnal (mean ± S.D. = 0.08 ± 0.12 $m^{-2}$) trawls (independent samples *t*-test, $t = 0.395$, $n = 160$, $P = 0.693$).

**Seasonal variations**

There were seasonal variations in the abundance of spined loach, with densities generally highest in the summer (June-August) and low in the autumn, winter and spring (Table 2; Kruskal-Wallis test, $K = 23.385$, $n = 172$, $P = 0.001$). In 2006, the density of spined loach in the River Trent satisfied the criterion for 'favourable condition' in June (mean ± S.D. = 0.11 ± 0.24 $m^{-2}$) and July (mean ± S.D. = 0.23 ± 0.49 $m^{-2}$), but not during the rest of the year (Table 2). There were also seasonal variations in the population structure of spined loach, with more age classes captured during the summer (June-August) than in the rest of the year, although there was no clear pattern in the percentage contribution of 0+ year individuals (Table 2; Figure 3). Despite the seasonal variations in the density and age structure of spined loach catches, there were no seasonal differences in the condition of the populations because at least one of the parameters always failed to satisfy the assessment criteria (Table 2).

**Annual variations**

There were annual variations in the autumn/winter abundance of spined loach. In the River Trent, densities were highest in 2009 and lowest in 2003, 2004, 2008 and 2011 (Kruskal-Wallis test, $K = 45.274$, $n = 250$, $P < 0.001$), whereas they were highest in 2008 and lowest in 2010 in the River Ancholme, although the differences were not statistically significant in the latter river (Kruskal-Wallis test, $K = 3.113$, $n = 91$, $P = 0.375$) (Table 3). There were also annual variations in the population structure of spined loach, but there was no apparent association between density, the number of age classes and the percentage contribution of 0+ year individuals (Table 3). Despite the variations in the density and age structure of spined loach catches, there were no annual differences in the condition of the populations because at least one of the parameters always failed to satisfy the assessment criteria (Table 3).

**Spatial variations**

There were also spatial variations in the abundance of spined loach. Densities in individual trawls ranged from 0 to 0.83 $m^{-2}$ in the Counter Drain and from 0 to 0.33 $m^{-2}$ in the Gravel Drain. The highest densities were recorded from the upstream (south-west) reach of the Counter Drain, with densities further downstream being low (Figure 4; Kruskal-Wallis test, $K$ = 29.514, $n$ = 50, $P$ < 0.001). The Counter Drain had a significantly higher mean (and maximum) density of spined loach than the Gravel Drain (Mann-Whitney $U$-test, $U$ = 1721.500, $n$ = 150, $P$ < 0.001), and the density exceeded that required to achieve 'favourable condition' in the Counter Drain (mean ± S.D. = 0.16 ± 0.24 $m^{-2}$), but not in the Gravel Drain (mean ± S.D. = 0.02 ± 0.06 $m^{-2}$). A minimum of three age classes of spined loach was captured from both drains, but there was a significant difference in their length distributions (two-sample Kolmogorov-Smirnov test, $Z$ = 1.995, $n$ = 64, $P$ = 0.001), with 0+ individuals comprising 62% and 24% of the catches in the Counter and Gravel Drains, respectively. The structure of the spined loach population satisfied the criteria to achieve 'favourable condition' (>2 age classes, >50% 0+ year individuals) in the Counter Drain, but not in the Gravel Drain. Moreover, there were differences in the condition assessment of the Counter Drain depending upon where the surveys were conducted: inclusion of sites 31-50 resulted in the condition being assessed as 'favourable', whereas surveys only at sites 1-30 resulted in 'unfavourable condition' (Table 4). Although not statistically different (Mann-Whitney $U$-test, $U$ = 454.000, $n$ = 166, $P$ = 0.755), the density of spined loach in trawl catches (mean ± S.D. = 0.065 ± 0.158 $m^{-2}$) was an order-of-magnitude higher than in seine catches (mean ± S.D. = 0.003 ± 0.005 $m^{-2}$).

## DISCUSSION

### Variations in abundance and population structure

All organisms are subject to spatio-temporal variations in abundance and population structure. Such phenomena occur naturally and, indeed, are fundamental to the processes driving biological diversity, community ecology and ecosystem functioning. Spatio-temporal variations in abundance and population structure also occur in scarce and rare species, making it difficult to set quantitative conservation targets, especially, as is the case in spined loach, if autecological knowledge or baseline data are limited.

Spined loach exhibited strong diel variations in abundance, with densities generally being low during the day and peaking at night. Indeed, the relative abundance of spined loach in diurnal samples was only 15% of nocturnal samples, and densities satisfied the criterion for 'favourable condition' at night, but not during the day. In addition, the poor diurnal catches of spined loach meant that fewer age classes were captured during the day than at night. This can probably be explained largely by the nocturnal behaviour of spined loach; peaks in activity, as well as changes in habitat use, have been observed at night (Culling *et al*., 2003; Marszal *et al*., 2003). The results of the current study indicate that spined loach were active in the shallow margins of the marina at night, but presumably sheltered in sediments or dense vegetation during daylight. This has important implications for the condition assessment and conservation of spined loach populations, because monitoring programmes conducted only during daylight, or using methods that are inefficient during daylight, are likely to underestimate the abundance and population structure of this nocturnal species. Further research into diel variations in the ecology, especially habitat use, of spined loach is required to facilitate the conservation of the species (Copp and Vilizzi, 2004).

There were seasonal variations in the abundance and population structure of spined loach, with densities and the number of age classes generally highest in the summer (June-August). Indeed, in 2006, the density of spined loach in the River Trent satisfied the criterion for ‘favourable condition’ in June and July, but not during the rest of the year. Spined loach spawn in early summer (Robotham, 1981; Bohlen, 1999, 2000b, 2003; Juchno & Boroń, 2006a, b), which will inevitably have an influence on their abundance, the number of age classes and the percentage contribution of 0+ year individuals. In addition, habitat use or characteristics may vary on a seasonal basis. For example, spined loach have been found to leave shallow margins in the autumn (Ritterbusch and Bohlen, 2000), and their distribution in the River Great Ouse appeared to be linked to seasonal variations in substratum composition (Robotham, 1978). The current monitoring protocol states that surveys should be conducted in the autumn/winter (Joint Nature Conservation Committee, 2005). This has important implications for the condition assessment and conservation of spined loach populations, because monitoring programmes conducted in the autumn/winter may underestimate their abundance and population structure, especially if conducted after the fish have left their shallow, summer habitats (Ritterbusch and Bohlen, 2000).

There were annual variations in the autumn/winter abundance and population structure of spined loach. A wide range of biotic (e.g. competition, predation, disease) and abiotic (e.g. climate, weather, physicochemistry, habitat) factors influence the population dynamics of fishes (Houde, 1987; Myers *et al*., 1997; Nunn *et al*., 2007, 2010, 2012; Longshaw *et al*., 2010). Little is known about the factors that affect the stability of spined loach populations, although those that affect other fish species are undoubtedly influential, and annual variations in abundance and population structure have been observed elsewhere (Slavík and Ráb, 1999; Ritterbusch and Bohlen, 2000). Annual variations in abundance and population structure have important implications for the condition assessment and conservation of spined loach populations, because the reporting frequency for SAC species (6 years) renders it difficult to assess the stability of their populations or detect the early signs of possible catastrophes.

The highest densities and numbers of age classes of spined loach were recorded from the upstream reach of the Counter Drain, with densities/numbers of age classes further downstream, and in the Gravel Drain, being low. Indeed, the mean density of spined loach in the Counter Drain would more than double if calculated using only the 20 most-upstream samples. Moreover, there were differences in the condition assessment of the Counter Drain depending upon where the surveys were conducted: inclusion of sites 31-50 resulted in the condition being assessed as ‘favourable’, whereas surveys only at sites 1-30 resulted in ‘unfavourable condition’. This was probably caused by spatial variations in physical habitat characteristics. Spined loach generally inhabit areas characterised by fine substratum containing organic components (Robotham, 1977, 1978; Slavík *et al*., 2000). Water velocity, filamentous algae and macrophytes can also be influential, and there may be inter-gender differences or ontogenetic shifts in microhabitat use (Bohlen, 2000a, b; Culling *et al*., 2003; Copp and Vilizzi, 2004). Water velocity was slow throughout the study area and mud was ubiquitous, but the

Counter Drain was generally wider and deeper than the Gravel Drain, and had a greater coverage of submerged macrophytes, filamentous algae and detritus (AD Nunn, unpubl. data). The highest densities of spined loach were recorded from the upstream reach of the Counter Drain, which was characterised by oxic, rather than anoxic, mud, extensive submerged macrophytes and relatively fast-flowing water, as well as *relatively* large and small, respectively, coverages of gravel and filamentous algae (AD Nunn, unpubl. data). Spatial variations in abundance and population structure have important implications for the condition assessment and conservation of spined loach populations, because monitoring programmes conducted in inappropriate areas (e.g. only unsuitable or optimal habitats) may underestimate, or overestimate, their status.

**Condition assessment and conservation**

Extremely small and isolated populations of a number of fish species have apparently persisted for centuries, possibly millennia, yet reliable estimates of minimum viable population sizes remain elusive (Gaston and Lawton, 1990; Maitland and Lyle, 1991; Traill *et al*., 2007). Similarly, it is unclear what constitutes a viable population in spined loach and, therefore, what criteria are suitable for condition assessment. Currently, any reduction in density results in an ‘unfavourable condition’ status, even if densities are historically high (Joint Nature Conservation Committee, 2005). Not only does this require baseline data against which to compare contemporary data, but densities naturally vary temporally; identifying when a reduction in density is a cause for concern is therefore problematic. Similarly, setting density thresholds is problematic because densities also naturally differ between habitats.

The selection of the thresholds for the condition assessment criteria (Joint Nature Conservation Committee, 2005) was rather arbitrary, although it is recognised that it was unavoidable given the lack of knowledge and baseline data on spined loach populations. In the current study, the mean (± S.D.) autumn/winter densities of spined loach were similar in the Trent (0.02 ± 0.02 $m^{-2}$), Ancholme (0.01 ± 0.01 $m^{-2}$) and Gravel Drain (0.02 ± 0.06 $m^{-2}$). Moreover, the densities were considerably lower than the threshold to achieve 'favourable' condition (0.1 $m^{-2}$), yet the continued presence of the species, the relatively stable densities over time (e.g. 1999-2012 in the Trent) and successful annual recruitment suggests that the populations are sustainable. Furthermore, diel, seasonal, annual and spatial variations in the density and age structure of spined loach catches had few impacts on the condition of their populations because at least one of the parameters invariably failed to satisfy the assessment criteria. It is therefore recommended that the condition assessment criteria are reviewed and amended by collating and analysing all available data on spined loach populations, as well as conducting further specific surveys to address knowledge gaps (Cowx *et al.*, 2009).

The number and size of samples will inevitably affect overall catches and, potentially, condition assessments. Another limitation of the current monitoring protocol (Joint Nature Conservation Committee, 2005) is that the condition assessment uses criteria based upon individual (density) *and* combined (number of age classes, percentage contribution of 0+ year age class) catches. Although abundance can be expressed per unit effort and averaged to account for the number and size of samples, the number of age classes detected is likely to increase with increasing numbers or sizes of samples if samples are combined for analysis. Guidance on the number and/or size of samples that should be collected and sampling strategies is therefore desirable in a revised monitoring protocol (see Cowx *et al*., 2009). In addition, for conservation purposes it may be important to identify and assess the condition of distinct populations or sub-populations. Exactly what constitutes a ‘population’ should therefore be defined in a revised monitoring protocol, together with guidance on how to account for spatial variations in abundance and population structure in condition assessments.

It should be borne in mind that different gears were used in the rivers and drains. Although seine nets sometimes caught large numbers of spined loach, especially in the Trent at night, it is recommended that trawling is used to conduct condition assessments because, unlike seine netting, its efficiency appears to be unaffected by the nocturnal behaviour of spined loach, as the gear effectively captures spined loach buried in sediment or vegetation. In addition, although not *statistically* significant, the density of spined loach in trawl catches was an order-of-magnitude higher than in seine catches, which could have significant implications for *condition assessment*. Trawling also avoids the logistical difficulties associated with conducting nocturnal seine surveys, as well as large bycatches of larval and juveniles fishes, and allows a large number of small samples to be collected, which is more statistically robust and provides more detailed biogeographical information than a small number of large (e.g. seine) samples (Copp, 2010). Large numbers of small samples should also maximise the range of microhabitats that are surveyed and, potentially, increase the number of age classes that are captured. The current monitoring protocol (Joint Nature Conservation Committee, 2005) states that trawling should be used in drains, whereas electric fishing should be used in rivers. Although electric fishing may be useful in some situations, in many areas the water will be either too deep, turbid or vegetated for efficient sampling, especially of 0+ year individuals. Similarly, the low percentage contribution of 0+ year individuals in many of the catches in this study probably reflects the inefficiency of the seine net at capturing such small fish (Cowx *et al*., 2001), particularly in dense macrophytes, where young spined loach tend to be found (Bohlen, 2000a, b).

A prerequisite for successful conservation is an effective monitoring programme. Monitoring programmes will only be effective if the chosen sampling strategies and methods are able to detect target species at low levels of abundance, to avoid underestimates of population status through imperfect detection (Kéry and Schmidt, 2008; Britton *et al*., 2011). Monitoring programmes must also be able to detect changes in temporal and spatial structure relating to species distributions and abundances (Cowx *et al*., 2009, 2010; Reynolds *et al*., 2011; Rolls *et al*., 2013). It is therefore essential that surveys to assess the condition of populations of designated species are designed with due consideration of their diel behaviour, breeding season, life span and habitat use. It is thus recommended that surveys for spined loach are conducted: (1) by trawling; (2) in late summer; and (3) at least every 3-4 years. It is also recommended that the influence of spatio-temporal variations in abundance and population structure, and of sampling strategies, methodologies and techniques, on the condition assessment of other species of conservation interest (e.g. Atlantic salmon (*Salmo salar* L.), lampreys, shads, bullhead (*Cottus gobio* L.); see Maitland and Lyle, 1991; Life in UK Rivers, 2003) should be rigorously evaluated, so that their respective monitoring protocols and/or condition assessment criteria can be amended if necessary.

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Table 1 Diel variations in the density, age structure and condition of the spined loach population in a boating marina on the River Trent, England.

| **Date** | **Period** | **Mean density (no. $m^{-2}$)** | **No. age classes** | **% 0+** | **Population condition** |
|---|---|---|---|---|---|
| 3-4 June 2009 | Day | 0.00 | 1 | 100* | Unfavourable |
| | Night | 0.08 | 3* | 78* | Unfavourable |
| 16-17 June 2009 | Day | 0.01 | 1 | 100* | Unfavourable |
| | Night | 0.08 | 3* | 82* | Unfavourable |
| 1-2 July 2009 | Day | 0.03 | 3* | 8 | Unfavourable |
| | Night | 0.26* | 3* | 0 | Unfavourable |
| 19-20 May 2010 | Day | 0.00 | 1 | 0 | Unfavourable |
| | Night | 0.03 | 2 | 17 | Unfavourable |
| 2-3 June 2010 | Day | 0.00 | 1 | 0 | Unfavourable |
| | Night | 0.02 | 1 | 100* | Unfavourable |
| 16-17 June 2010 | Day | 0.16* | 1 | 100* | Unfavourable |
| | Night | 0.70* | 2 | 99* | Unfavourable |
| 30 June-1 July 2010 | Day | 0.03 | 2 | 38 | Unfavourable |
| | Night | 0.90* | 3* | 14 | Unfavourable |
| 14-15 July 2010 | Day | 0.13* | 3* | 41 | Unfavourable |
| | Night | 0.36* | 3* | 12 | Unfavourable |

Parameters satisfying the respective condition assessment criterion (mean density >0.10 $m^{-2}$, >2 age classes, >50% 0+ year individuals; Joint Nature Conservation Committee, 2005) are asterisked, nocturnal surveys are shaded.

Table 2 Seasonal variations in the density, age structure and condition of the spined loach population in the River Trent, England, in 2006.

| **Month** | **Mean density (no. $m^{-2}$)** | **No. age classes** | **% 0+** | **Population condition** |
|---|---|---|---|---|
| January | 0 | 0 | 0 | Unfavourable |
| February | 0 | 0 | 0 | Unfavourable |
| March | 0 | 0 | 0 | Unfavourable |
| April | <0.01 | 2 | 62* | Unfavourable |
| May | 0.01 | 3* | 53* | Unfavourable |
| June | 0.11* | 3* | 9 | Unfavourable |
| July | 0.23* | 3* | 34 | Unfavourable |
| August | 0.05 | 3* | 58* | Unfavourable |
| September | 0.01 | 2 | 43 | Unfavourable |
| October | <0.01 | 1 | 100* | Unfavourable |
| November | 0 | 0 | 0 | Unfavourable |

Parameters satisfying the respective condition assessment criterion (mean density >0.10 $m^{-2}$, >2 age classes, >50% 0+ year individuals; Joint Nature Conservation Committee, 2005) are asterisked, fish from the 2005 year class were aged as 0+ year individuals until the appearance of the 2006 year class.

Table 3 Annual variations in the autumn/winter density, age structure and condition of the spined loach populations in the River Trent, River Ancholme and River Glen Counter and Gravel Drains, England.

| **River/Drain** | **Year** | **Mean density (no. $m^{-2}$)** | **No. age classes** | **% 0+** | **Population condition** |
|---|---|---|---|---|---|
| Trent | 1999 | 0.02 | 3* | 12 | Unfavourable |
| | 2000 | <0.01 | 1 | 100* | Unfavourable |
| | 2001 | 0.02 | 3* | 27 | Unfavourable |
| | 2002 | 0.02 | 1 | 0 | Unfavourable |
| | 2003 | 0 | 0 | 0 | Unfavourable |
| | 2004 | 0 | 0 | 0 | Unfavourable |
| | 2005 | 0.01 | 3* | 73* | Unfavourable |
| | 2006 | 0.02 | 3* | 53* | Unfavourable |
| | 2007 | 0.03 | 2 | 18 | Unfavourable |
| | 2008 | 0 | 0 | 0 | Unfavourable |
| | 2009 | 0.06 | 2 | 9 | Unfavourable |
| | 2010 | 0.02 | 2 | 64* | Unfavourable |
| | 2011 | 0 | 1 | 0 | Unfavourable |
| | 2012 | <0.01 | 1 | 0 | Unfavourable |
| Ancholme | 2008 | 0.02 | 3* | 67* | Unfavourable |
| | 2009 | 0.01 | 3* | 34 | Unfavourable |
| | 2010 | <0.01 | 2 | 17 | Unfavourable |
| | 2011 | 0.01 | 3* | 29 | Unfavourable |
| Counter | 2012 | 0.16* | 3* | 62* | Favourable |
| Gravel | 2012 | 0.02 | 3* | 24 | Unfavourable |

Parameters satisfying the respective condition assessment criterion (mean density >0.10 $m^{-2}$, >2 age classes, >50% 0+ year individuals; Joint Nature Conservation Committee, 2005) are asterisked.

Table 4 Spatial variations in the density, age structure and condition of the spined loach population in the River Glen Counter Drain, England, in October 2012.

| **Site no.** | **Mean density (no. $m^{-2}$)** | **No. age classes** | **% 0+** | **Population condition** |
|---|---|---|---|---|
| 1-10 | 0 | 0 | 0 | Unfavourable |
| 1-20 | 0.01 | 1 | 100* | Unfavourable |
| 1-30 | 0.03 | 2 | 60* | Unfavourable |
| 1-40 | 0.13* | 3* | 59* | Favourable |
| 1-50 | 0.16* | 3* | 62* | Favourable |
| | | | | |
| 11-20 | 0.02 | 1 | 100* | Unfavourable |
| 11-30 | 0.04 | 2 | 60* | Unfavourable |
| 11-40 | 0.18* | 3* | 59* | Favourable |
| 11-50 | 0.20* | 3* | 62* | Favourable |
| | | | | |
| 21-30 | 0.07 | 2 | 50 | Unfavourable |
| 21-40 | 0.26* | 3* | 61* | Favourable |
| 21-50 | 0.26* | 3* | 61* | Favourable |
| | | | | |
| 31-40 | 0.45* | 3* | 63* | Favourable |
| 31-50 | 0.35* | 3* | 60* | Favourable |
| | | | | |
| 41-50 | 0.25* | 3* | 60* | Favourable |

Parameters satisfying the respective condition assessment criterion (mean density >0.10 $m^{-2}$, >2 age classes, >50% 0+ year individuals; Joint Nature Conservation Committee, 2005) are asterisked.

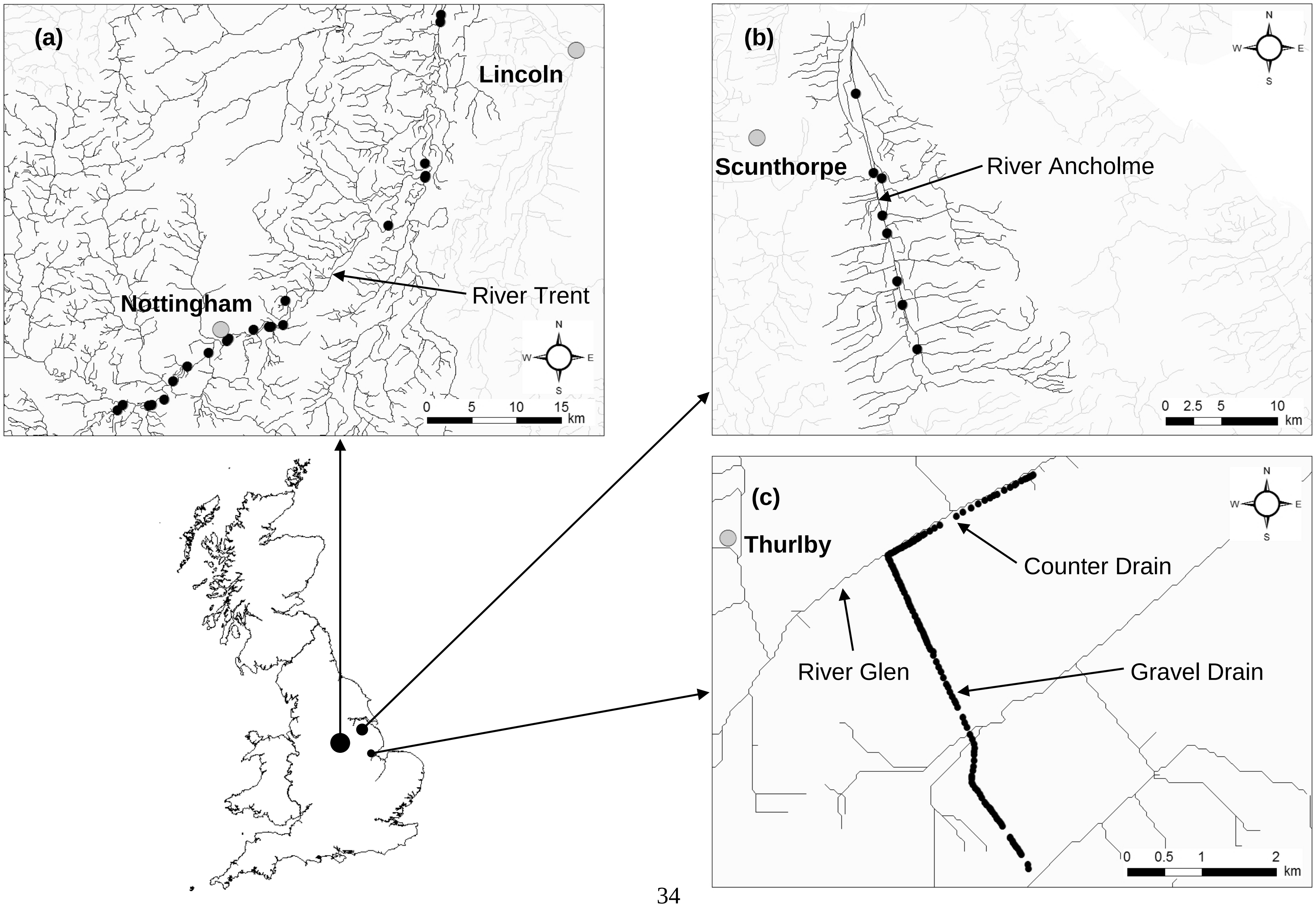
## FIGURE LEGENDS

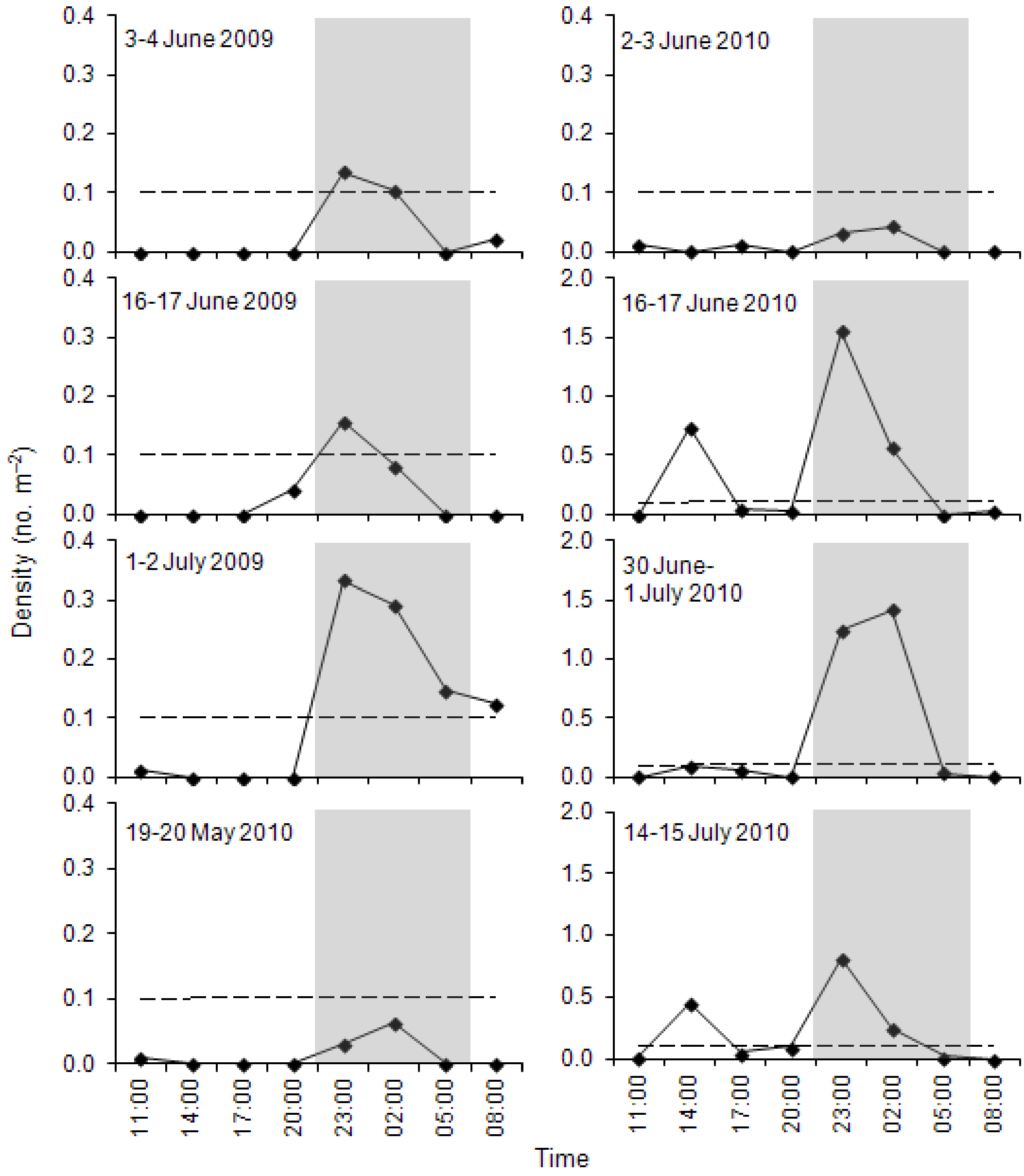
Figure 1 Locations of the survey sites on the (a) River Trent, (b) River Ancholme and (c) River Glen Counter and Gravel Drains, England.

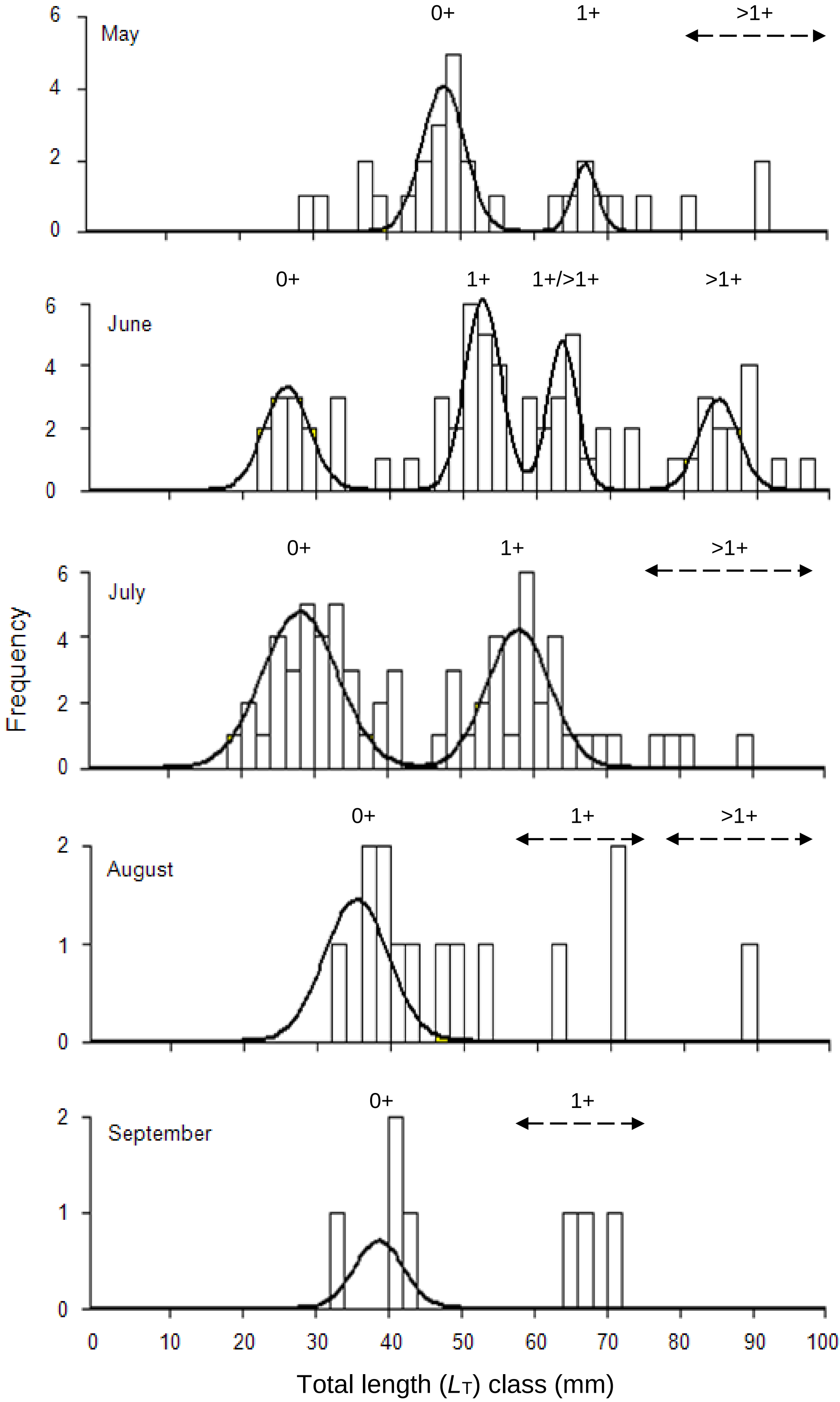
Figure 2 Diel variations in the density (no. $m^{-2}$) of spined loach in a boating marina on the River Trent, England. Nocturnal samples are shaded, and the density required to achieve 'favourable condition' is indicated by the dashed line.

Figure 3 Seasonal variations in the population structure of spined loach in the River Trent, England, in 2006. Modal groups (≈ age classes) were identified using modal progression analysis when possible, otherwise the approximate length ranges of the age classes are illustrated. Fish from the 2005 year class were aged as 0+ year individuals until the appearance of the 2006 year class. There must be at least three age classes and a high percentage, preferably at least 50%, of 0+ year individuals to achieve 'favourable condition' (Joint Nature Conservation Committee, 2005).

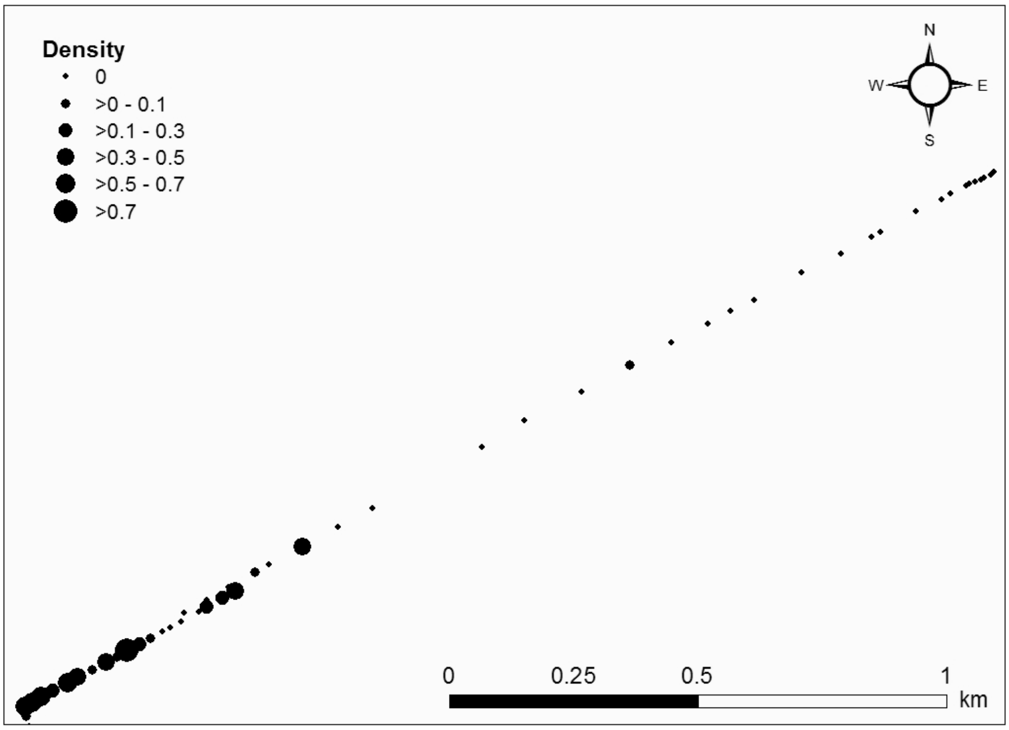
Figure 4 Spatial variations in the density (no. $m^{-2}$) of spined loach in the River Glen Counter Drain, England, in October 2012. The drain flows in a north-easterly direction. Densities must be >0.1 $m^{-2}$ to achieve 'favourable condition' (Joint Nature Conservation Committee, 2005).


(a)
Lincoln
Nottingham
River Trent
N
W
E
S
0 5 10 15
km
(b)
Scunthorpe
River Ancholme
N
W
E
S
0 2.5 5 10
km
(c)
Thurlby
Counter Drain
River Glen
Gravel Drain
N
W
E
S
0 0.5 1 2
km


3-4 June 2009
2-3 June 2010
16-17 June 2009
16-17 June 2010
1-2 July 2009
30 June-
1 July 2010
19-20 May 2010
14-15 July 2010
Density (no. m-2)
Time
11:00
14:00
17:00
20:00
23:00
02:00
05:00
08:00
0.0
0.1
0.2
0.3
0.4
0.5
1.0
1.5
2.0



May
June
July
August
September
0+
1+
>1+
1+/>1+
Frequency
0
2
4
6
1
0
10
20
30
40
50
60
70
80
90
100
Total length ($L_T$) class (mm)


Density
0
>0 - 0.1
>0.1 - 0.3
>0.3 - 0.5
>0.5 - 0.7
>0.7
N
W
E
S
0
0.25
0.5
1
km